

CLAIMS

1. Method for preparing a gamma delta T lymphocytes composition, comprising at least one step of culturing a biological preparation comprising at least 50 million mononuclear
5 cells in the presence of a synthetic activator compound of gamma delta T lymphocytes at initiation of the culture, followed by culture in the presence of a cytokine.
2. Method according to claim 1, wherein the biological preparation is a blood, plasma or serum sample.
- 10 3. Method according to claim 2, wherein the biological preparation is from a cytapheresis.
4. Method according to any one of claims 1 to 3, wherein the biological preparation comprises more than $10 \cdot 10^7$ cells.
- 15 5. Method according to any one of claims 1 to 4, wherein the biological preparation has previously been frozen.
6. Method according to any one of claims 1 to 5, wherein the cells are maintained during
20 culture at a density less than about $5 \cdot 10^6$ cells/ml.
7. Method according to any one of claims 1 to 6, wherein the cells are cultured for a time period greater than or equal to about 10 days, preferably between 10 and 25 days.
- 25 8. Method according to any one of the previous claims, wherein the synthetic activator compound of gamma delta T lymphocytes is a ligand of the T cell receptor of gamma delta T lymphocytes.
9. Method according to claim 8, wherein the synthetic activator compound of gamma delta
30 T lymphocytes is selected in the group consisting of phosphohalohydrin compounds, phosphoepoxide compounds and bisphosphonate compounds.

10. Method according to claim 9, wherein the synthetic activator compound of gamma delta T lymphocytes is selected in the group consisting of the following compounds:

3-(bromomethyl)-3-butanol-1-yl-diphosphate (BrHPP)

5 3-(iodomethyl)-3-butanol-1-yl-diphosphate (IHPP)

3-(chloromethyl)-3-butanol-1-yl-diphosphate (ClHPP)

3-(bromomethyl)-3-butanol-1-yl-triphosphate (BrHPPP)

3-(iodomethyl)-3-butanol-1-yl-triphosphate (IHPPP)

α,γ -di-[3-(bromomethyl)-3-butanol-1-yl]-triphosphate (diBrHTP)

10 α,γ -di-[3-(iodomethyl)-3-butanol-1-yl]-triphosphate (diIHTP)

3,4,-epoxy-3-methyl-1-butyl-diphosphate (EpoX-PP)

3,4,-epoxy-3-methyl-1-butyl-triphosphate (EpoX-PPP)

α,γ -di-3,4,-epoxy-3-methyl-1-butyl-triphosphate (di-EpoX-TP)

15 11. Method according to any one of the previous claims, wherein the cytokine is selected in the group consisting of interleukin-2 and interleukin-15.

12. Method according to any one of the previous claims, wherein the cytokine is used at a concentration comprised between about 150 U/ml and about 500 U/ml.

20 13. Method according to any one of the previous claims, wherein the resulting composition has the following characteristics :

- it comprises more than 80 % gamma delta T cells, and

- it comprises more than 100 million viable and functional gamma delta T cells.

25 14. Method for preparing a cell composition comprising functional gamma delta T lymphocytes, wherein it comprises at least :

. culturing cells from a cytopheresis in the presence of a synthetic activator compound of gamma delta T lymphocytes and a cytokine selected in the group consisting of interleukin-

30 2 and interleukin-15, said culture being carried out in conditions ensuring that cell density is maintained essentially below $5 \cdot 10^6$ cells/ml, and

. recovering some or all of the cells obtained, said cells comprising functional gamma delta T lymphocytes.

5 15. Method for preparing a pharmaceutical composition based on gamma delta T lymphocytes, the method comprising :

- . culturing cells according to the method described in any one of claims 1 to 14,
- . recovering some or all of the cells obtained, said cells comprising functional gamma delta T lymphocytes, and
- . formulating the cells in a pharmaceutically acceptable carrier or excipient.

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16. Pharmaceutical composition, wherein it comprises a population of cells composed of more than 80 % functional gamma delta T lymphocytes and comprising more than 100 million gamma delta T lymphocytes.

15 17. Composition according to claim 16, wherein it additionally comprises human serum albumin.

20 18. Composition according to any one of claims 16 or 17, wherein it additionally comprises a cytokine preferably selected in the group consisting of IL-2 and IL-15, in view of use simultaneously, separately or spread out over time.

19. Culture of blood cells *in vitro* or *ex vivo*, wherein it comprises at least 80 % functional gamma delta T lymphocytes and more than 100 million gamma delta T lymphocytes.

25 20. Use of a cell culture according to claim 19 for preparing a pharmaceutical composition for stimulating the immune defenses of a subject, particularly for treating infectious or parasitic diseases or cancers.